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Rhamnolipids: essential virulence factors for early invasion of primary human airway epithelia by *Pseudomonas*

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The opportunistic bacteria *Pseudomonas aeruginosa* (*Pa*) cause chronic respiratory infections in cystic fibrosis. However, the mechanism by which the pathogen initially modulates the paracellular permeability of polarized respiratory epithelia to access the basolateral domain of the cells remains to be understood, as a prerequisite to develop novel therapeutic strategies. We have searched for virulence factors secreted by *Pa*, other than elastase and LPS, that could affect the structure of human airway epithelium in the early stages of infection.
We have found that 1) only bacterial strains capable of secreting rhamnolipids efficiently down regulate the barrier function of an *in vitro* reconstituted human respiratory epithelium, even in the absence of elastase; 2) purified rhamnolipids, applied on the surface of the epithelia, are sufficient to promote the paracellular invasion of *Pa*, even of strains deficient in the quorum sensing systems; 3) at variance with previous reports, *Pa* remains exclusively in the paracellular space of the epithelia; 4) fluorescent rhamnolipids interact with the apical cell membrane, inducing a specific alteration of ciliated cells, between which pathogens infiltrate and 5) blockade of this interaction prevents early *Pa* infection.
The study provides direct evidence for a hitherto unknown mechanism whereby the junction-dependent barrier of the respiratory mucosa is selectively altered by rhamnolipids, thereby accounting for its invasion by *Pa*.
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Garlic which blocks the *P. aeruginosa* QS systems, promotes rapid clearing of pulmonary *P. aeruginosa* infections

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The opportunistic human pathogen *Pseudomonas aeruginosa* is the predominant microorganism of chronic lung infections in cystic fibrosis patients. Quorum sensing (QS) renders the biofilm growing bacteria highly tolerant to otherwise lethal doses of antibiotics, and protect from the bactericidal activity of polymorphonuclear leukocytes (PMNs). We have previously demonstrated that QS is inhibited by garlic extract. In this study we have evaluated the effect of garlic for the interaction between *P. aeruginosa* and tobramycin and the PMNs and outcome of pulmonary infection.
P. aeruginosa was grown as biofilms with and without garlic extract. Biofilms were treated with tobramycin or PMNs. The garlic treated biofilm was susceptible to both tobramycin and PMNs. The PMNs showed an increased activation, when incubated on the garlic treated biofilm visualised by staining the PMNs with 123-DHR, which is green flouresent when H_2O_2 is produced - respiratory burst. The differences in sensitivity were observed by confocal microscopy of "live- dead" stained biofilms. Mice were treated with garlic extract or saline for 7 days, 2 day profylactic and infected with *P. aeruginosa*. Bacteriology, mortality, histopathology, and cytokines were endpoints.
The results indicate that a QS inhibitory extract of garlic renders *P. aeruginosa* sensitive to both tobramycin and, phagocytosis, and respiratory burst of polymorphonuclear neutrophile leukocytes. Garlic treatment of a pulmonary mouse model initially provokes a higher degree of inflammation and improved clearing of the infecting bacteria.

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Factors influencing adenovirus-mediated airway transduction in fetal mice

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Fetal gene therapy for the treatment of cystic fibrosis has several advantages over similar treatment in the adult. Treatment before birth may allow avoidance of *in utero* pathology and may allow permanent correction of the genetic defect by transduction of fetal stem cells. Intra-amniotic injection of marker/vector allows access to the fetal airways following natural fetal breathing movements. This administration method is promising for use in gene therapy for cystic fibrosis where the main target for exogenous gene expression is the lung. Here, we have investigated factors that may affect the efficacy of gene transfer to the murine fetal lung. We examined marker compound distribution and transgene expression (from a first generation adenoviral vector) at different stages of fetal development. This demonstrated that fetal breathing movements at 15-16 days of gestation are of sufficient intensity to carry marker/vector into the fetal lungs. These movements can be significantly stimulated by the combination of intra-amniotic theophylline administration and post-operative exposure of the dam to elevated CO_2 levels. However, the most important factor for efficient and consistent pulmonary transgene delivery is the dose of adenoviral vector used, as both the degree of transduction and the percentage of lungs transduced increases with escalating viral dose. The optimization of intra-amniotic injections will be very useful in future studies using more appropriate vectors in CF mouse models.

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Investigation of the role of quorum sensing in biofilm formation by *B. cenocepacia* strains

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The *Burkholderia cepacia* complex (BCC) has emerged as multiple antibiotic resistant bacteria, resulting in persistent chronic infection and reduced life expectancy in cystic fibrosis (CF) patients. Little is understood about the mechanisms involved in *B. cepacia* biofilm formation, its role in CF pathogenesis and whether cell-cell communication via a quorum-sensing (QS) system is involved. In this study we determined the ability of BCC strains to generate and develop biofilms and investigated the role of the *cep* QS system in BCC biofilm formation. Biofilm formation was tested in 96-well plates using 17 BCC strains taken from the *B. cepacia* experimental strain panel (designated by the International *B. cepacia* Working Group) and representative of eight species. Strains from *B. multivorans* and *B. cenocepacia*, the more clinically relevant strains, formed biofilms more readily as determined by a two fold increase in crystal violet intensity than strains from *B. cepacia*, *B. stabilis* or *B. vietnamiensis* and the recently identified species, *B. dolosa*, *B. ambifaria* and *B. pyrrocinia*. Mutants deficient in functioning *cepI* and *cepR* genes for *B. cenocepacia* strains J2315 and BC7 were generated. Using these QS mutants we found that *B. cenocepacia* biofilm formation requires a functional *cepIR* QS system. This confirms that QS has a key role in BCC biofilm formation and hence contributes to the virulence of BCC. The QS system of BCC therefore is a potential target for the development of novel therapeutic strategies in the treatment of BCC infected patients.
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